VARIETAL DIFFERENCES IN COLORANT PROPERTIES AND STABILITY OF RED BEET PIGMENTS

4402

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ABSTRACT

Samples of 48 beet cultivars were screened for differences in pigment content and stability. Large differences were found between cultivars in betacyanine content and in the proportion of betacyanines and betaxanthines. Extensive variability in pigment content was seen within samples. The thermal stability of betacyanines in diluted beet juice at 100°C was not subject to varietal effects. Differences in betacyanine stability at 25°C between cultivars were significant at the 0.10 level, but not at 0.05. Betacyanines were less stable at pH 3 than at pH 5 and were highly light sensitive. Betaxanthines were similarly affected by pH and light; betaxanthines were much less stable than betacyanines at 25°C.

INTRODUCTION

THE USE OF pigments from red beets (*Beta vulgaris*) as a food colorant has attracted considerable interest in recent years because of the need to replace banned red food dyes (von Elbe, 1975). A number of ingredient suppliers are developing beet juice concentrates and powders for this purpose. Such products would be substantially more costly and less satisfactory with respect to color and stability than the FD&C dyes that they would replace (Riboh, 1977).

Beets contain two distinct classes of pigments, the purple betacyanines and the yellow betaxanthines, referred to collectively as betalaines. Varietal differences in the composition, spectral properties and color of betalaines have been reported for a small number of beet cultivars (Aronoff and Aronoff, 1948; Peterson and Joslyn, 1960; von Elbe et al., 1972). Knowledge of such differences would be of value in the selection of beet varieties for the commercial production of a food colorant. A study was undertaken by our laboratory in cooperation with the USDA North Central Regional Plant Introduction Station in Ames, IA, to develop such information.

EXPERIMENTAL

Raw material

A collection of 325 accessions of *Beta vulgaris*, representing many Asiatic and European beet cultivars, was grown at the Regional Plant Introduction Station at Ames, IA during the 1977 season. The collection was screened in late August by visual inspection of longitudinal cross sections of typical roots for pigmentation, and 36 highly pigmented accessions were selected for further study. Two weeks after the initial screening, samples were harvested, topped and shipped by air freight to our laboratory for further evaluation. On arrival, the beets were washed, air-dried, and packaged in polyethylene bags. Samples were stored at −18°C until December when they could be analyzed.

In addition to these samples, a 17-accession collection of domestic and European beet cultivars, grown by the W. Atlee Burpee Company, at its Doylestown, PA, experiment station, also was screened, and 12 cultivars were selected for study. Samples comprising several dozen beet roots of each cultivar were harvested in late September and brought to our laboratory for evaluation. Portions of each sample, required for the measurement of colorant properties, were topped and stored at 1°C until analyses could be completed (1–2 wk). The remaining roots, required for the measurement of pigment stability, were topped, cleaned and packaged in the same manner as the Iowa samples and were stored at −18°C until November when these studies could be carried out.

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Evaluation of colorant properties

Refrigerated beet samples were removed from storage, washed and air-dried. Prewashed frozen samples were held under refrigeration until thawed. Five to seven typical roots from each sample were then cut in half along the longitudinal axis for visual observation of root-to-root variability. Atypical roots were noted and removed from the sample if greatly different from the remaining roots. Root halves were further subdivided so that a representative composite sample weighing 400–500g could be prepared. After each composite sample was weighed, the beet juice was extracted with an Acme Supreme Juicerator Model 6001 (Acme Juicer Manufacturing Co., Lemoyne, PA) lined with a 6.3 × 56.6 cm strip of Whatman No. 1 filter paper. After extraction, the juice volume was measured, and the juice was stirred to assure uniformity.

The soluble solids content of beet juice samples was determined with a Bausch and Lomb Abbe-3L Refractometer. The juice was then diluted 1/100 with McIlvaine's buffer at pH 3.0. Absorbance measurements were made against a buffer blank at 537 nm with a Bausch and Lomb Spectronic 88 Spectrophotometer and between 375 and 650 nm with a Bausch and Lomb Spectronic 505 Recording Spectrophotometer. A second pH 3.0 dilution of each beet juice sample, having an absorbance of 1.0 at 537 nm, was prepared for analysis with the Gardner XL-23 Tristimulus Colorimeter. This concentration adjustment simplified the direct comparison of samples, which varied widely in pigment content and color. A 50-ml aliquot was added to a clear glass optical cell, 57.1 mm in diameter (ID) and 40 mm in height, to a depth of 20 mm. Samples were analyzed in the transmission mode, and values of the Hunter L, a_L and b_L coordinates were obtained. Turbidity in diluted beet juice, which was encountered with some samples, was removed by filtration through Whatman No. 2 filter paper preceding these optical measurements.

Estimates of betacyanine and betaxanthine concentration in the beet juice samples were obtained from the spectrophotometric data by the method of Saguy et al. (1978); computations were based on absorbance values taken at 10-nm intervals between 375 and 650 nm.

Root-to-root variability in betacyanine content

Root-to-root variability in the betacyanine content of the Burpee samples was determined by extracting the juice from each of five individual preweighed roots and measuring the absorbance at 537 nm of a 1/100 dilution prepared with pH 3.0 buffer. The betacyanine content of juice samples was calculated from the spectrophotometric data and the extinction coefficient for betanine ($E^{1\,cm}_{1\%}$ = 1120). Means for each cultivar were compared by Duncan's multiple range test.

Evaluation of heat stability

The heat stability of betacyanine in beet juice samples, diluted 1/100 with pH 3.0 or pH 5.0 buffer, was determined in air at 100°C. Duplicate 75 × 12 mm test tubes containing 3.0 ml of diluted juice were immersed in a boiling water bath for 1–5 min and then cooled rapidly by immersion in an ice bath for 2 min. Betacyanine was determined spectrophotometrically at 537 nm. First-order rate constants were obtained from the plotted data.

Evaluation of storage stability

The stability of betacyanine in sterile beet juice, diluted with pH 3.0 or pH 5.0 buffer, was determined during storage in air at 25°C with samples exposed to light and to the dark. Enzymes in beet juice samples that could degrade betacyanine during storage were inactivated by boiling the juice for 2 min. The heated juice samples

Table 1—Pigment and soluble solids content of 20 red beet cultivars

Cultivar	Source and accession no.[a]	Betacyanine[b] (mg/100g beets)	Betaxanthine[b] (mg/100g beets)	Betaxanthine-betacyanine ratio	Soluble solids (%)
Uniball	Burpee (Netherlands)	38.7	20.6	0.53	11.1
Slowbolt R-2289	Burpee (Denmark)	35.8	15.1	0.42	9.3
Red E403	Burpee (Denmark)	34.9	19.3	0.56	8.8
Bordo 237	PI293420 (USSR)	34.6	21.9	0.63	9.2
Detroit Nero RS	Burpee (Netherlands)	33.2	16.2	0.49	8.0
Detroit Dark Red MT	Burpee (USA)	32.0	11.5	0.36	9.0
Detroit Dark Red ST	Burpee (USA)	31.6	13.5	0.43	9.2
Podzimniaja 0474	PI293419 (USSR)	31.3	15.0	0.48	8.3
Detroit Sluis	Burpee (Netherlands)	30.7	17.3	0.56	10.0
Early Wonder	Burpee (USA)	30.4	13.2	0.43	10.2
Little Ball	Burpee (Netherlands)	30.3	11.1	0.37	7.0
Choghundur	PI163179 (India)	30.2	18.2	0.60	7.2
Iowa	IA CHeck	28.3	14.9	0.53	7.4
Gladiator	Burpee (USA)	27.9	12.0	0.43	10.9
Asmer Beethoven	Burpee (England)	27.8	10.8	0.39	9.2
Crveno	PI357355 (Yugoslavia)	27.1	15.2	0.56	9.6
Polsko	PI357351 (Yugoslavia)	26.9	16.7	0.62	8.2
Okragly Ciemnoczerwony	PI285591 (Poland)	25.8	11.4	0.44	9.0
Spangsbjerg	PI269310 (Sweden)	25.2	16.7	0.66	8.0
Rubidus	Burpee (Netherlands)	23.5	9.6	0.41	7.9

[a] Grown in Doylestown, PA, (Burpee) or in Ames, IA (PI (Plant Introduction) accessions)
[b] Calculated as betanine or vulgaxanthine by the method of Saguy et al. (1978).

were immediately cooled in a refrigerator and then filtered through Whatman No. 2 paper to remove coagulated solids. The filtrate was diluted with McIlvaine buffer at pH 3.0 or 5.0 to yield a solution having an absorbance at 537 nm of about 1.0; dilutions varied from 2/100 to 4/100, depending on the pigment content of the juice. The diluted juice was sterilized by filtration through a disposable Falcon 0.22 micron membrane filter unit under vacuum; 15-ml aliquots of sterile diluted juice were pipetted aseptically with a Brewer Automatic Pipetting Machine (Model 40) into sterile 125 × 16 mm screw-cap test tubes having Teflon®-lined caps. Test tubes and pipetting machine parts in contact with the samples were sterilized by heating in an autoclave at 15 psi for 30 min. The filled test tubes were stored horizontally in the dark or on a white table under fluorescent lighting (G.E. Staybright Cool White Tubes, Model F40CW/S) producing 90–100 footcandles of illumination at the table surface. Duplicate tubes were taken for spectrophotometric analysis at 537 nm after storage for 1–14 days. First-order rate constants for betacyanine degradation during storage were obtained by regression analysis of the spectrophotometric data. Rate constants for the degradation of betaxanthines in sterile beet juice dilutions during storage were obtained by a similar procedure, betaxanthine and betacyanine concentrations in these samples being computed by the method of Saguy et al. (1978) as described previously.

RESULTS & DISCUSSION

Pigment content and colorant properties of beet cultivars

The betacyanine, betaxanthine, and soluble solids contents of the 20 most highly pigmented cultivars in our study are given in Table 1. The other 28 cultivars screened were substantially lower in pigment content and are not included in the table. The pigment content of each sample, calculated as mg betacyanine or betaxanthine per 100g beets, is an indication of pigment yield. On this basis, the betacyanine content varied between 23.5 and 38.7 mg/100g beets for the top 20 cultivars and was as low as 2.7 mg per 100g beets for the least pigmented cultivar in the study. A range of 36–135 mg betacyanine per 100g was reported by von Elbe et al. (1972) for four breeding lines of beets.

Pigment contents can also be calculated on the basis of the juice soluble solids content, i.e., mg pigment per 100g soluble solids. This value would be indicative of the tinctorial strength of beet juice powder made from the sample. The soluble solids content of the cultivars compared in Table 1 varied between 7.0 and 11.1%. Expressed on a juice solids basis, the betacyanine content of these cultivars was between 385 and 706 mg per 100g for Gladiator and Little Ball, respectively.

The betaxanthine content of the 20 most highly pigmented cultivars in this study varied between 9.6 and 21.9 mg/100g beets. The betaxanthine content was also expressed as a fraction of the betacyanine content since this ratio appeared to be related to the beet juice color, samples with high ratios being more orange-red and samples with low ratios being more purple. Values of the betaxanthine-betacyanine ratio varied between 0.36 and 0.63 for the 20 most highly pigmented cultivars. A much wider range, 0.13-1.25, was found for the 28 less highly pigmented cultivars not shown in Table 1. A low betaxanthine-betacyanine ratio would be desirable in a betalaine-based food colorant to minimize color shifts during thermal processing or storage because of differential changes in the two pigment classes as reported recently by Savolainen and Kuusi (1978).

Beet juice samples to be analyzed by tristimulus colorimetry (light transmission mode) were standardized by dilution to an absorbance of 1.0 at 537 nm so that cultivars could be compared at the same L-value (Table 2). The tristimulus coordinate a_L, a measurement of sample redness, varied over a relatively narrow range, being slightly lower in samples having higher b_L values. The tristimulus coordinate b_L, a measurement of sample yellowness (+) or blueness (−), varied over a wider range, 8.5–17.2 for the 20 most highly pigmented cultivars and −15.0 to 20.9 for all cultivars in the study. A high positive correlation (r = 0.89) was obtained between b_L and the ratio of betaxanthines (yellow) to betacyanines (purple). A related parameter, the hue angle ($\tan^{-1} b_L/a_L$) and the betaxanthines-betacyanines ratio also were correlated (r= 0.86). Values of the hue angle were between −10.3 (purple) and 15.2 (red) for all cultivars in the study. Differences between samples in saturation index $(a_L^2 + b_L^2)^{1/2}$, a measure of color purity, were small. This is a consequence of the relative constancy and large magnitude of the a_L^2 term in the saturation index function.

Varietal differences in the betacyanine content and betaxanthine-betacyanine ratio of beet cultivars are sufficiently great to suggest the development of new cultivars bred specifically for their pigment content. Ideally, such

Table 2—Hunter color transmission values for standardized dilutions of juice from 20 red beet cultivars

Cultivar	Source and accession no.[a]	L	a_L	b_L	Hue angle $\tan^{-1}\frac{b_L}{a_L}$	Saturation index $(a_L^2 + b_L^2)^{1/2}$
Uniball	Burpee (Netherlands)	38.9	72.4	14.4	11.2	73.8
Slowbolt R-2289	Burpee (Denmark)	39.0	70.9	11.6	9.3	71.8
Red E403	Burpee (Denmark)	38.2	67.7	17.2	14.2	69.8
Bordo 237	PI293420 (USSR)	38.8	70.6	16.0	12.8	72.4
Detroit Nero RS	Burpee (Netherlands)	38.4	70.0	13.5	10.9	71.3
Detroit Dark Red MT	Burpee (USA)	38.7	71.6	10.3	8.2	72.3
Detroit Dark Red ST	Burpee (USA)	37.8	68.2	13.5	11.2	69.5
Podzimniaja 0474	PI293419 (USSR)	39.3	73.0	11.8	9.2	73.9
Detroit Sluis	Burpee (Netherlands)	37.8	66.8	15.1	12.7	68.5
Early Wonder	Burpee (USA)	37.7	69.9	11.8	9.6	70.9
Little Ball	Burpee (Netherlands)	39.2	70.8	8.5	6.8	71.3
Choghundur	PI163179 (India)	38.1	70.3	15.7	12.6	72.0
Iowa	IA Check	38.4	70.9	13.0	10.4	72.1
Gladiator	Burpee (USA)	38.8	71.2	11.3	9.0	72.1
Asmer Beethoven	Burpee (England)	37.0	66.5	11.4	9.7	67.5
Crveno	PI357355 (Yugoslavia)	37.9	68.7	14.9	12.2	70.3
Polsko	PI357351 (Yugoslavia)	37.6	69.0	14.6	11.9	70.5
Okragly Ciemnoczerwony	PI285591 (Poland)	38.6	72.3	9.5	7.5	72.9
Spangsbjerg	PI269310 (Sweden)	37.6	68.1	16.9	13.9	70.2
Rubidus	Burpee (Netherlands)	38.0	69.0	11.7	9.6	70.0

[a] Grown in Doylestown, PA, (Burpee) or in Ames, IA, (PI (Plant Introduction) accessions)

cultivars would be high in betacyanine, low in betaxanthine and low in soluble solids content. Of the 48 cultivars screened in this study, Little Ball and Detroit Dark Red MT came closest to meeting these criteria.

The pigment content of beets varies, not only between cultivars, but also within cultivars. In this study, the juice of individual beets in some samples grown in Doylestown varied in betacyanine content over a twofold range (Table 3). These cultivars differed greatly in variability; values of the coefficient of variation were between 4.3 and 36.5%. Variability in betacyanine content generally was not related to root weight, although with 7 of the 12 varieties, the largest beet in each sample was lower in betacyanine than were the other beets. Kaack (1977) has reported a decrease in betanine content during growth of red beets that could be described by an exponential function. Root-to-root differences in pigment content are a reflection of physiological factors controlling betacyanine biosynthesis and degradation. Wohlpart and Black (1973) observed that betanine formation in leaf discs of *B. vulgaris,* cultured on agar media, was stimulated by light and sucrose. Conceivably, the betacyanine content of beet roots could be optimized by manipulation of cultural or storage conditions so as to favor pigment biosynthesis.

Root-to-root variability within cultivars could complicate their selection for use as a colorant source. To be significantly different (5% level), the samples described in Table 3 would have to differ in betacyanine content by 26 mg per 100g, on a beet-juice basis, or by 16 mg per 100g on a whole-beet basis. Applying the latter value to the data in Table 1, we conclude that the 20 most highly pigmented cultivars were not significantly different in betacyanine content. However, differences in betacyanine content between these cultivars and the 28 less highly pigmented cultivars would be significant.

Pigment stability

Our measurements of betacyanine retention in diluted beet juice during heating at 100°C were consistent with previous reports that the thermal degradation of beet pigments follows first-order kinetics and is strongly pH-dependent (von Elbe et al., 1974; Savolainen and Kuusi, 1978; Saguy et al., 1978). Therefore, we based our evaluation of varietal differences in pigment stability on the comparison of first-order rate constants obtained with 26 cultivars at two pH values, 3 and 5. Differences in thermal stability between cultivars were not statistically significant. Rate constants were threefold higher at pH 3 ($k = 0.37 \pm 0.02\ min^{-1}$) than at pH 5 ($k = 0.11 \pm 0.01\ min^{-1}$). Similar pH effects were reported by von Elbe et al. (1974), but their values of the first-order rate constants for beet juice were much smaller than rate constants calculated from our data. This difference may be due to the loss of the protective effect reported by von Elbe et al. (1974) when beet juice is diluted. We have observed large differences in heat stability between diluted and undiluted beet juice, the latter having a degradation rate ($k = 0.038\ min^{-1}$) similar to von Elbe's.

The degradation of betacyanine in diluted beet juice during storage at 25°C followed first-order kinetics and was strongly dependent on pH and exposure to light, as has been reported previously by von Elbe et al. (1974). A comparison of degradation rate constants obtained with 30 cul-

Table 3—Root-to-root variability in betacyanine content of red beet cultivars

Cultivar	Betacyanine in juice (mg/100g)[a] Mean[b]	Range	Largest beet	Std dev	Coeff. of var. (%)
Detroit Dark Red MT	84.2a	62.3–106.0	71.4	18.0	21.4
Little Ball	70.2ab	50.0– 97.0	77.0	17.9	25.6
Gladiator	69.0ab	55.5– 82.9	68.9	11.4	16.5
Detroit Dark Red ST	68.0ab	53.6– 82.1	53.6	10.1	14.9
Red E403	66.8ab	57.8– 87.3	57.8	10.7	16.0
Rubidus	65.7ab	41.9– 93.7	41.9	17.1	26.1
Slowbolt R-2289	64.6ab	44.6– 85.0	49.4	16.7	25.8
Early Wonder	62.6ab	39.0–105.4	39.0	22.8	36.5
Detroit Nero RS	58.7b	39.4– 69.2	66.2	11.3	19.3
Uniball	54.8b	44.6– 60.7	60.7	6.0	10.9
Asmer Beethoven	54.4b	43.7– 63.2	43.7	7.1	13.0
Detroit Sluis	54.2b	50.6– 57.5	52.8	2.3	4.3

[a] Calculated as betanine

[b] Means followed by the same letter or letters are not significantly different at the 0.05 level by Duncan's multiple range test

Table 4—Stability of beet root betacyanines at 25° C

	First order rate constant ($days^{-1}$)			
	pH 3		pH 5	
Cultivar	Light	Dark	Light	Dark
Red E 403	0.73	0.19	0.14	0.10
Asmer Beethoven	0.69	0.15	0.12	0.08
Mean of 30	0.35	0.14	0.11	0.07
Range	0.21–0.73	0.10–0.25	0.08–0.14	0.04–0.10
Std dev	0.13	0.04	0.02	0.01

Table 5—Stability of beet root betaxanthines and betacyanines at 25° C

	First-order rate constant ($days^{-1}$)			
	pH 3		pH 5	
Pigment[a]	Light	Dark	Light	Dark
Betaxanthines	3.2	1.4	0.52	0.40
Betacyanines	0.28	0.10	0.061	0.058

[a] In diluted beet juice

tivars revealed a small varietal effect at pH 3 in light (Table 4). Rate constants were significantly greater at the 0.10 level (but not the 0.05 level) for two cultivars, Red E 403 and Asmer Beethoven; these constants were 0.73 and 0.69, respectively. None of the cultivars possessed exceptionally favorable stability characteristics.

von Elbe et al. (1974) also found that betanine degradation during storage was pH and light dependent and followed first-order reaction kinetics. They reported a smaller light effect than that observed in our study, but their data were obtained at a lower temperature (15°C) and at a higher pH (7.0) than we used. Their value of the reaction rate constant at 25°C for betanine degradation at pH 5 was substantially greater than our value (0.88 $days^{-1}$ vs 0.066 $days^{-1}$) obtained with diluted beet juice stored in the dark. The discrepancy may be due to the protective effect of beet juice solids in our system which von Elbe et al. noted in their study with beet juice, beet puree, and other food systems.

During storage at 25°C, the color of diluted beet juice samples rapidly became more purple as the intensity decreased. Examination of visible absorption spectra obtained at intervals during the storage of a typical sample revealed that betaxanthines were disappearing more rapidly than betacyanines. First-order rate constants for pigment degradation, calculated from the spectrophotometric data by the method of Saguy et al. (1978), confirmed this observation (Table 5). Savolainen and Kuusi (1978) reported similar values of the degradation rate constant for vulgaxanthine-I at 25°C (3.02 and 0.49 $days^{-1}$ for pH 3 and pH 5 solutions, respectively). Betaxanthines and betacyanines were less stable in light than in the dark at pH 3; both pigments were less stable at pH 3 than at pH 5. Betaxanthine degradation was virtually complete within 24 hr at the lower pH. For this reason, food colorants derived from beets should be low in betaxanthines. Different shades of red could be produced in the absence of the yellow betaxanthine component by combining betacyanines with annatto, as has been suggested by Pasch et al. (1975).

CONCLUSIONS

LARGE VARIETAL DIFFERENCES occur in the betacyanine and betaxanthine contents of red beet root. Differences in pigment content also occur within cultivars, some cultivars being more variable than others.

The thermal stability of betacyanines in diluted beet juice at 100°C is similar for all cultivars in our study. First-order degradation rate constants are higher at pH 3 than at pH 5. Betacyanine degradation at 25°C follows first-order kinetics; rate constants are greater in light than in darkness and at pH 3 than at pH 5. Betacyanines are more stable than betaxanthines at 25°C. Varietal effects on storage stability are small.

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